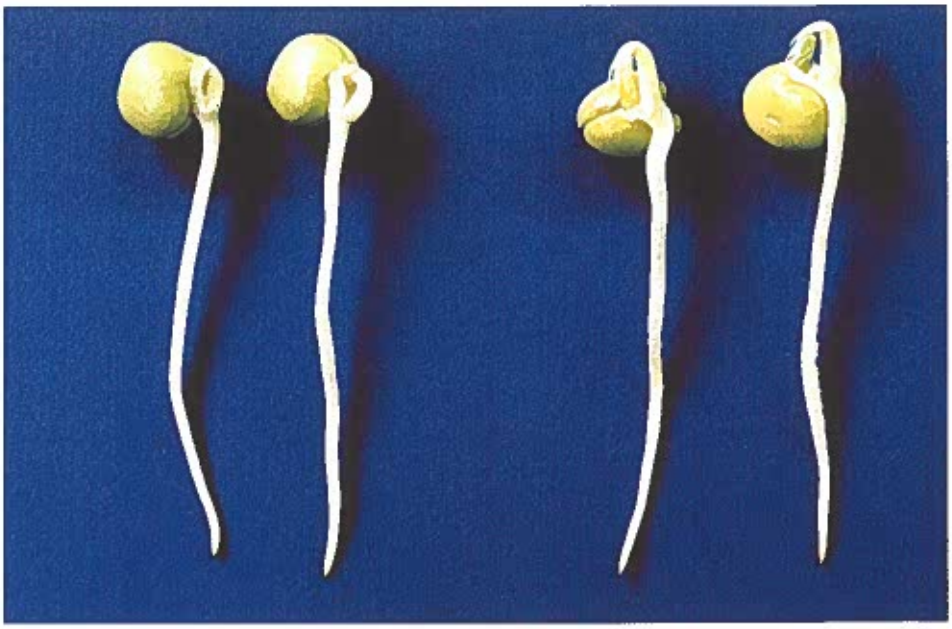
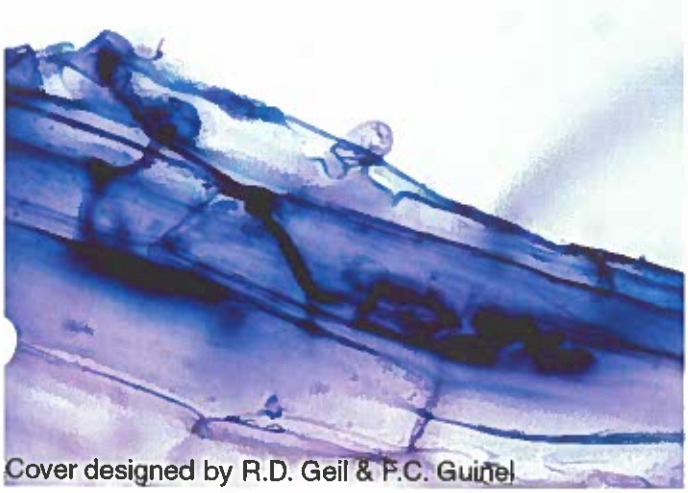
# 35th Plant Development Workshop

## Program & List of Contributors



November 3rd
2001

Department
of Biology



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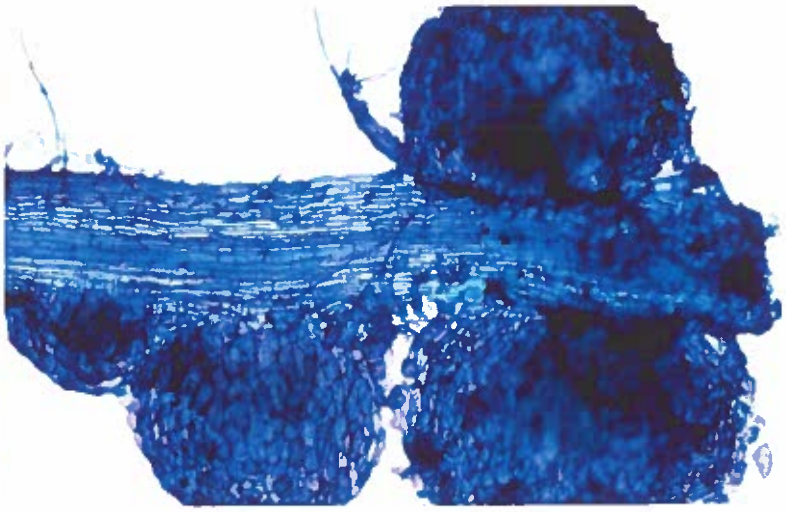

# 35th Plant Development Workshop

Saturday November 3, 2001

Organized by: Frédérique Guinel and Ryan Geil

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# List of Participants

| | | | | |
|---|---|---|---|---|
| Akhter | Sajjad | SRAKHTER@HOTMAIL.COM | Dept. of Biology | University of Waterloo |
| Amyot | Lisa | amyotl@EM.AGR.CA | SCPFRC | Agriculture and Agri-Food Canada |
| Buhlers | Deborah | dbuhlers@hotmail.com | Dept. of Biology | University of Waterloo |
| Chik | Halina | hhchik@scimail.uwaterloo.ca | Dept. of Biology | University of Waterloo |
| Cholewa | Ewa | cholewaewa@hotmail.com | Dept. of Biology | University of Waterloo |
| Dengler | Nancy | dengler@botany.utoronto.ca | Dept. of Botany | University of Toronto |
| Emery | Neil | nemery@trentu.ca | Biology Department | Trent University |
| Ferguson | Brett | bferguson34@hotmail.com | Dept. of Biology | Wilfrid Laurier University/ Univ. of Waterloo |
| Fieldes | Mary Ann | mfieldes@mach1.wlu.ca | Dept. of Biology | Wilfrid Laurier University |
| Gatsukovich | Yulia | ygatsuko@scimail.uwaterloo.ca | Dept. of Biology | University of Waterloo |
| Geil | Ryan | ryan_geil@hotmail.com | Dept. of Biology | Wilfrid Laurier University |
| Grbic | Vojislava | vgrbic@uwo.ca | Dept. of Plant Sciences | University of Western Ontario |
| Griffith | Marilyn | griffith@sciborg.uwaterloo.ca | Dept. of Biology | University of Waterloo |
| Guinel | Frederique | fguinel@wlu.ca | Dept. of Biology | Wilfrid Laurier University |
| Gunawardena | Arunika | arunika@hotmail.com | Dept. of Agricultural Biology | University of Peradeniya |
| Harvey | Chris | CGHarvey@Scimail.Uwaterloo.ca | Dept. of Biology | Wilfrid Laurier University/ Univ. of Waterloo |
| Horton | Roger | rhorton@uoguelph.ca | Dept. of Botany | University of Guelph |
| Kalinina | Anna | akalinina@devbiol.zoo.uwo.ca | Dept. of Plant Sciences | University of Western Ontario |
| Kang | Julie | kang@botany.utoronto.ca | Dept. of Botany | University of Toronto |
| Kotsovska | Olena | okotsovska@devbiol.zoo.uwo.ca | Dept. of Plant Sciences | University of Western Ontario |
| Kozela | Chris | ChrisKozela@pigeon.carleton.ca | Dept. of Biology | Carleton University |
| Lin | Lan | linl@mcmaster.ca | Dept. of Biology | McMaster |
| Lott | John | lott@mcmail.mcmaster.ca | Dept. of Biology | McMaster |
| Ma | Fengshan | mabiology@hotmail.com | Dept. of Plant Biology | Ohio State University |
| Massicotte | Hugues | hugues@unbc.ca | Forestry Department | University of Northern British Columbia |
| McDonnell | Lisa | lmcdonne@chat.carleton.ca | Dept. of Biology | Carleton University |
| McKown | Athena | twinflower@hotmail.com | Dept. of Biological Sciences | University of Alberta |
| Moffatt | Barb | moffatt@sciborg.uwaterloo.ca | Dept. of Biology | University of Waterloo |
| Murray | Jeremy | jeremymu@uoguelph.ca | Dept. of Plant Agriculture | University of Guelph |
| Nowack | Linda | lmnowack@scimail.uwaterloo.ca | Dept. of Biology | University of Waterloo |
| Oaks | Ann | aoaks@uoguelph.ca | Dept. of Botany | University of Guelph |
| Peirson | Dave | dpeirson@mach2.wlu.ca | Dean's Office | Wilfrid Laurier University |
| Pereira | Luiz | laperei@scimail.uwaterloo.ca | Dept. of Biology | University of Waterloo |
| Perry | Greg | geperry@scimail.uwaterloo.ca | Dept. of Biology | University of Waterloo |
| Peterson | Carol | cpeterso@uwaterloo.ca | Dept. of Biology | University of Waterloo |
| Poduska | Branislava | bpoduska@uwo.ca | Dept. of Plant Sciences | University of Western Ontario |
| Posluszny | Usher | uposlusz@uoguelph.ca | Dept. of Botany | University of Guelph |
| Regan | Sharon | Sharon_Regan@carleton.ca | Dept. of Biology | Carleton University |
| Soros | Connie | csoros@uoguelph.ca | Dept. of Botany | University of Guelph |
| Szczyglowski | Krzysztof | szczyglowskik@em.agr.ca | SCPFRC | Agriculture and Agri-Food Canada |
| Tackaberry | Linda | tackabl@unbc.ca | Forestry Department | University of Northern British Columbia |
| Talent | Nadia | nadia.talent@utoronto.ca | Dept. of Botany | University of Toronto |
| Tao | Wenjing | w2tao@sciborg.uwaterloo.ca | Dept. of Biology | University of Waterloo |
| Todorova | Margarita | mtodorov@sciborg.uwaterloo.ca | Dept. of Biology | University of Waterloo |
| Walsh | Kimberly | | Dept. of Plant Sciences | University of Western Ontario |
| Wilson | Trevor | trwilson@uoguelph.ca | Dept. of Botany | University of Guelph |

# 35th Plant Development Workshop

**Department of Biology**
**Wilfrid Laurier University**
**Science Building**

**8:30 am** **Registration**, Science Building Atrium

**9:00 am** **Welcoming Remarks**: Dr. David Peirson, Room N1044

**Contributed Oral Presentations**, Room N1044
Sessional Chair: Brett Ferguson

**9:15** Fengshan Ma and **Carol Peterson**
Frequencies and physiological significance of plasmodesmata in onion roots

**9:30** **Fengshan Ma**, Asuka Itaya, Yoshie Matsuda, Genqing Liang, Biao Ding
Protein transport in tobacco: the phloem and beyond

**9:45** **Linda Nowack**, Ewa Madey, Yulia Gatsukovich, John Thompson
A possible role for phloem fatty acids in long-distance signaling

**10:00** **Julie Kang**
The role of cell cycling during vascular development of *Arabidopsis* leaves

**10:15** **Ewa Cholewa**, Bjorn Sundberg
*Arabidopsis thaliana* as a model for studying secondary xylem development

**10:30 – 11:00 am** **Coffee Break**, Room N1046

**Contributed Oral Presentations**, Room N1044
Sessional Chair: Chris Harvey

**11:00** **Luiz Pereira**, Margarita Todorova, Barbara Moffatt, Christopher Makaroff
Changes in *S*-adenosylhomocysteine hydrolase and adenosine kinase expression, content and activity during flower and silique development in *Arabidopsis*

**11:15** **Branislava Poduska**, Antje Redweik, Vojislava Grbic
Genetic dissection of Sy-0, late-flowering aerial rosette bearing *Arabidopsis* ecotype

**11:30** Tai Wai Yeo, **Olena Kotsovska**, Vojislava Grbic
Analysis of Li:3-3, late flowering aerial rosette bearing *Arabidopsis* ecotype

**11:45 am – 2:15 pm** **LUNCH**, Room N1046 and Poster viewing, Atrium
**12:45 – 1:15** Presenters with **odd** numbers be at your posters
**1:30 – 2:00** Presenters with **even** numbers be at your posters

**Contributed Oral Presentations**, Room N1044
Sessional Chair: Ryan Geil

**2:15** **Jeremy Murray**, J. Larsen, T. Michaels, A. Schaafsma, C. Vallejos, K. Pauls
Gene discovery in bean (*Phaseolus vulgaris*) genomic (Bng) RFLP clones and observations on synteny between *P. vulgaris* and *Arabidopsis thaliana*

**2:30** **Anna Kalinina**, Nela Mihajlovic, Vojislava Grbic
The role of LEAFY in the initiation of the lateral primordia and activation of axillary meristems

**2:45** **Arunika Gunawardena**, M. Naganathan
Investigation of root anatomy of rice (*Oryza sativa*) and maize (*Zea mays*) grown in aerated and stagnant conditions

**3:00** **Trevor Wilson**, Usher Posluszny
A comparative study in morphology between Boston ivy (*Parthenocissus tricuspidata*) and Virginia creeper (*Parthenocissus quinquefolia*)

**3:15** **Roger Horton**
Making ethylene

**3:30 – 4:00 pm** **Coffee Break**, Room N1046

**4:00** Guest Speaker, Room 1044
**Krzysztof Szczyglowski** and Lisa Amyot
Symbiotic and non-symbiotic development in the model legume *Lotus japonicus*

**4:55** **Business Meeting**, Room N1044

**5:00 – 6:30 pm** **Wine and Cheese Reception**, Grad House

# Oral Presentation Abstracts

Fengshan Ma[1] and **Carol A. Peterson**[2]
[1]Department of Plant Biology, Ohio State University, Columbus, OH, USA, 43210
[2]Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, cpeterso@uwaterloo.ca

**Frequencies and physiological significance of plasmodesmata in onion roots**

Plasmodesmata in roots are important for the inward movement of ions and the outward movement of assimilates. The ease of movement of these substances within the symplast is related to the frequencies of functional plasmodesmata between cells. Using the onion root with a mature exodermis as a model system, the frequencies of plasmodesmata between all living cells were determined. (This is the first study in which such an analysis has been accomplished for an entire organ.) All cells of the root are connected to their neighboring cells by plasmodesmata, with the exception of the long cells of the exodermis and the mature tracheary elements. The plasmodesmata originally present in the former cells are severed by the development of suberin lamellae. The frequencies of plasmodesmata are very low between the epidermis and the short cells of the exodermis, and between the pericycle and neighboring cells of the stele. These results suggest that some ions move from the walls into the symplast in the short cells of the exodermis, and confirm the idea of Vakhmistrov that the pericycle can act as a preferential annular pathway for the movement of both ions and assimilates.

**Fengshan Ma**, Asuka Itaya, Yoshie Matsuda, Genqing Liang, and Biao Ding
Department of Plant Biology and the Plant Biotechnology Center, The Ohio State University, 1060 Carmack Rd., Columbus, OH, 43210, mabiology@hotmail.com

**Protein transport in tobacco: the phloem and beyond**

Transgenic plants of tobacco (*Nicotiana tabacum*) are used to study protein transport in the leaf and stem. One of the transgenic lines expresses the fusion protein 3a movement protein:GFP under the control of the comellina yellow mottle virus promoter which is companion cell-specific. Fluorescence microscopy, confocal laser scanning microscopy and immuno-gold transmission electron microscopy were used to monitor the localization and movement of the 3a:GFP protein. In the leaf veins of the 3a:GFP plants, the fusion protein targets to plasmodesmata and moves from companion cells (the sites of synthesis) to other cells inside the phloem, but rarely to cells beyond the phloem. In petioles and stems, by contrast, the protein can move out of the phloem. The fluorescent dye, fluorescein, when loaded into the phloem, exhibited the least movement capacity; that is, it just stays in the sieve element-companion cell complexes. These findings raise profound questions concerning the performance of the phloem and the mechanism(s) in transferring micro- and macro-molecules in different organs.

**Linda M. Nowack**, Ewa Madey, Yulia Gatsukovich, and John E. Thompson
Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, lmnowack@scimail.uwaterloo.ca

### A possible role for phloem fatty acids in long-distance signaling

A novel class of lipid-containing particles has been isolated from phloem sap of canola. The particles are spherical, and of variable size. The lipid composition of the particles is markedly different from that of microsomal membranes and cytosol isolated from both leaves and stems. Specifically, the phloem particles contain predominantly unesterified fatty acids and are enriched in medium-chain fatty acids, in particular, lauric ($C_{12:0}$), myristic ($C_{14:0}$) and pentadecanoic ($C_{15:0}$) acids. This unique lipid profile distinguishes phloem particles from other plant lipid bodies. Furthermore, the phloem content of unusual fatty acids increases significantly in plants exposed to sublethal levels of UV radiation stress. The greatest effect is observed in the levels of lauric acid, which increase from 3.4 % to 24.1% of total fatty acid pool. The presence of lipid particles in the phloem sap suggests long-distance transport of lipid in plants, and their enrichment in unusual medium chain fatty acids implies their potential involvement in signaling events. This notion is further supported by the rapid increase in lauric acid in phloem sap in response to a stress stimulus. We propose that free lauric acid in phloem may participate in signal transduction via N-acylphosphatidylethanolamine (NAPE) metabolism, known to be upregulated in response to pathogen elicitors.

**Julie Kang**
Department of Botany, University of Toronto, Toronto, ON, M5S 3B2, kang@botany.utoronto.ca

### The role of cell cycling during vascular development of *Arabidopsis* leaves

The cell cycle plays several distinct roles during plant development. One of these roles is during histogenesis, the process by which cells differentiate from undifferentiated precursors. Cell cycling is involved in at least three different aspects of histogenesis: 1) pattern formation, 2) cell proliferation, and 3) cell differentiation. One aspect that has not yet been thoroughly studied is what role the cell cycle plays during vascular development. Using a *cyc1At*::GUS construct, I examined the expression patterns of cell proliferation in procambial strands during leaf development. As well, I have used the *Athb-8*::GUS construct to examine the development of vein pattern, since the *Athb-8* gene is one of the earliest known markers of procambial strand formation. Based on the expression patterns of *cyc1At*::GUS and *AtHB-8*::GUS, I have found that the frequency of cell cycling differed among vein orders and that vein pattern was formed very early in leaf development, well before the pattern of protoxylem differentiation.

While the basic cell cycle machinery has been well conserved between plants and animals, how this machinery is regulated during plant development is still largely unknown. A number of genes have been found to play a role in regulating the cell cycle. One gene, *Aintegumenta-1 (ANT-1),* has been shown to regulate cell proliferation in *Arabidopsis* leaves. Using the *ANT1*::GUS reporter construct, I have found that *ANT1*::GUS is expressed in procambial cells during leaf development. Based on these results, I hypothesize that *ANT1* may play a role in regulating vascular pattern formation, in addition to general cell proliferation in the leaf. This study will provide an understanding of vascular pattern formation and regulation of cell proliferation during vascular development.

**Ewa Cholewa**[1] and Bjorn Sundberg[2]
[1] Department of Biology, University of Waterloo, ON, N2L 3G1, cholewaewa@hotmail.com
[2] Department of Forest Genetics and Plant Physiology, SLU, Umea, Sweden

### *Arabidopsis thaliana* as a model for studying secondary xylem development

Compared to the abundance of studies on primary meristems, the vascular and cork cambia have been virtually unexplored in *Arabidopsis*. The usefulness of the *Arabidopsis* as a model system to investigate basic questions about secondary xylem development dependents upon the anatomical characteristics of its secondary tissues. We present here analyses of the development of wild-type Columbia plants, accompanied with detailed description of development of secondary xylem and phloem throughout a period of two months. In *Arabidopsis*, vascular cambium develops and undergoes secondary growth at the bottom of the inflorescences, at the branching points of the secondary inflorescences, in the hypocotyls, and in the main roots. The initiation of vascular cambium first occurs in hypocotyls where the divisions of the procambial cells takes place 6 to 7 days after germination and it is correlated with the development of the first true leaves. The secondary xylem of the hypocotyls develops in two phases, an early phase in which only vessel elements mature, and a later stage in which both vessel elements and fibres are present. The fascicular and interfascicular cambia develop in the inflorescence a complete ring of secondary tissues composed of vessels and fibres in the fascicular regions and fibres only between the fascicles. The lignification of the fibres in the inflorescence is very rapid and precedes the lignification of the fibres in hypocotyls. We have evaluated a number of staining techniques and developed a new double-staining technique with TBO in 20% $CaCl_2$ and ruthenium red for fast detailed observations of the lignified secondary and cambial primary cell walls simultaneously in the same section. Reporting the growth stage during which data were obtained can provide an explicit developmental context for comparative purposes of mutants in the determination of gene function.

**Luiz Pereira**, Margarita I. Todorova, Barbara A. Moffatt, and Christopher A. Makaroff
Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, laperei@scimail.uwaterloo.ca

### Changes in *S*-adenosylhomocysteine hydrolase and adenosine kinase expression, content and activity during flower and silique development in *Arabidopsis*

During plant development a large amount of methyl groups are required for the synthesis or modification of many compounds such as DNA, RNA, phospholipids, proteins, pectin and lignin. The major methyl donor for these reactions is *S*-adenosylmethionine (SAM). Each SAM-dependent methylation produces a molecule of *S*-adenosyl homocysteine (SAH), which is a strong competitive inhibitor of all SAM-dependent transmethylases reactions. Consequently, SAH must be continually removed to prevent such inhibition. We are studying the contribution of two enzymes *S*-adenosylhomocysteine hydrolase (SAHH) and adenosine kinase (ADK) to the utilization and recycling of SAM, during flower and silique development in Arabidopsis. For this purpose the expression patterns, protein levels and activities of SAHH and ADK have been investigated in these organs by in situ hybridization, northern and western analysis and in vitro enzyme assays. In flowers, SAHH and ADK transcript highest in carpels, floral meristems, ovules, filaments and pollen. SAHH transcript levels are low during the initial stages of silique development, rapidly increase prior to the dehiscence zone formation and then drastically drop. The enzymatic activities vary during silique development in a pattern that corresponds in most of the cases to the transcripts levels of SAHH and ADK. These results are discussed with respect to the methylation demand of these organs during development.

**Branislava Poduska**, Antje Redweik, and Vojislava Grbic
Department of Plant Sciences, University of Western Ontario, London, ON, N6A 5B8, bpoduska@uwo.ca

**Genetic dissection of Sy-0, late-flowering aerial rosette bearing *Arabidopsis* ecotype**

Sy-0 ecotype has delayed transition to reproductive development of all shoot meristems resulting in an enlarged basal rosette, in formation of aerial rosettes and in conversion of early flowers into shoots. Dominant alleles of four genes are required for the Sy-0 phenotype. They map at two locations in a genome: FRI on chromosome IV, and ART1 and FLC map within 20cM on chromosome V. Currently the map position of ART2 is uncertain. ART1 is newly identified flowering locus that together with ART2 activates FLC in FRI-independent manner. ART1 and ART2 also act synergistically with FRI to delay flowering, independently from FLC. Complex of genes on chromosome V is epistatic to late-flowering mutants disrupting the autonomous and photoperiod-sensing pathway, however, EMF and LFY are epistatic to the ART complex. The ART1 locus has been localized to a 15kb fragment containing 3 ORFs with following putative functions: a proteinase, an unknown protein and transcription factor.

Tai Wai Yeo, **Olena Kotsovska**, and Vojislava Grbic
Department of Plant Sciences, University of Western Ontario, London, ON, N6A 5B8, okotsovska@devbiol.uwo.ca

**Analysis of Li:3-3, late-flowering aerial rosette bearing *Arabidopsis* ecotype**

Flowering time is regulated by complex genetic network that integrates environmental cues and developmental state of the plant. To elucidate the molecular mechanism of flowering in Arabidopsis we investigate the genetic bases underlying the formation of aerial rosettes in a subset of the late-flowering Arabidopsis ecotypes.

Li:3-3 is a late-flowering aerial rosette-bearing Arabidopsis ecotype. Delayed transition to reproductive development of Li:3-3 plants can be suppressed by vernalization, and transition to reproductive development can be completely abolished if plants are grown under short photoperiod. These physiological responses suggest that Li:3-3 carries genes that act through the autonomous flowering pathway. Analyses of the F2 and BC1 segregation populations indicate that dominant alleles of two unlinked loci are responsible for the aerial rosette phenotype. These loci have been tentatively identified as new alleles of FRI and FLC late-flowering genes.

**J. Murray**[1], J. Larsen[1], T.E. Michaels[1], A. Schaafsma[2], C.E. Vallejos[3], and K.P. Pauls[1]
[1]Department of Plant Agriculture, University of Guelph, Guelph, ON, N1G 2W1, jeremymu@uoguelph.ca
[2]Ridgetown College of Agricultural Technology, Ridgetown, ON
[3]Department of Vegetable Crops, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida

**Gene discovery in bean (*Phaseolus vulgaris*) genomic (Bng) RFLP clones and observations on synteny between *P. vulgaris* and *Arabidopsis thaliana***

A set of 87 previously-mapped bean (*Phaseolus vulgaris*) genomic (Bng) clones were partially sequenced. BLAST database searches detected homologies between 59 of these clones and genes from a variety of plants, especially *Arabidopsis thaliana*, *Glycine max* and *Medicago truncatula*. Sequence from several of these clones matched that of known genes including a lon protease, an early nodulin binding protein, a vacuolar sorting receptor as well as four genes involved in sulfur metabolism. Comparisons of the order of Bng clones on a *Phaseolus* linkage map with the positions of the corresponding homologs on the *Arabidopsis* AGI map revealed several regions of synteny, including a 57 cM region on *P. vulgaris* linkage group G (Florida map) and *Arabidopsis* chromosome V. One clone belonging to this group, Bng94, contained sequence that was homologous to PCMP genes. This large gene family is unique to plants and its homologs cluster with NBS-LRR resistance gene analogs in *Arabidopsis*.

**Anna Kalinina**, Nela Mihajlovic, and Vojislava Grbic
Department of Plant Sciences, University of Western Ontario, London, ON, N6A 5B8, akalinina@devbiol.zoo.uwo.ca

**The role of LEAFY in the initiation of the lateral primordia and activation of axillary meristems**

At transition to reproductive development several processes occur concomitantly. First, the primary shoot apical meristem ceases initiation of leaves and commences initiation of floral meristems; second, axillary meristems initiate at the axils of youngest leaf primordia in a basipetal pattern, and third, the rate of organ initiation increases. Simultaneous occurrence of these processes in Arabidopsis raise the possibility that they are coordinately regulated.

LFY, a transcriptional factor, promotes floral meristem development through activating floral meristem identity genes. This study uses lfy 1, a loss-of-function allele, and gain-of-function 35S-LFY construct to investigate whether LFY also plays a role in establishment of the elevated rate of primordia initiation and activation of axillary meristems. Our results indicate that LFY may be required for maintenance of the high rate of primordia initiation under SD photoperiod and that LFY, together with the independently acting factor, can activate axillary meristem development.

**H. L. A. N. Gunawardena** and M. Naganathan
Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, SRI LANKA, arunika@hotmail.com, arunika@agbio.pdn.ac.lk

### Investigation of root anatomy of rice (*Oryza sativa*) and maize (*Zea mays*) grown in aerated and stagnant conditions

Aerenchyma is a tissue type, which facilitates gas diffusion between roots and the aerial environment. The main barriers to water flow in monocot roots are sclerenchymatous layer, casparian bands and suberin lamella in the endodermis and exodermis.

Maize and rice roots were grown hydroponically in the incubator at 25 $^0$C. Roots lengths were measured every day and marked at a distance of 10 mm from the root tip every 24 hrs. Free hand sections were taken and stained with Sudan 7B and Berberine aniline blue.

Initial results showed that root growth was high under aerated condition in maize compared to the stagnant condition over time. However, in rice, root growth was more or less similar in aerated and stagnant conditions. Formation of aerenchyma was observed in maize grown in stagnant conditions, but not in aerated condition. In rice aerenchyma was observed in both aerated and stagnant conditions. Initiation of aerenchyma was observed in the mid cortex region.

The barriers to water flow such as casparian bands and suberin lamella in endodermis and exodermis were prominent in maize under stagnant condition. In rice, in addition to suberized endodermis and exodermis suberization and casparian bands, very thick sclerenchyma layer also were present under stagnant condition.

**Trevor C. Wilson** and Usher Posluszny
Department of Botany, University of Guelph, Guelph, ON, N1G 2W1, trwilson@uoguelph.ca

### A comparative study in morphology between Boston ivy *(Parthenocissus tricuspidata)* and Virginia creeper *(Parthenocissus quinquefolia)*

The developmental morphology of *Parthenocissus tricuspidata* (Boston Ivy) and *Parthenocissus quinquefolia* (Virginia Creeper) was compared. The two species are distinguished from their relatives in the Vitaceae (the Grape Family) by their adhesive tendrils. Microscopic techniques have been used to observe developmental stages of a tendril from inception to maturation. The addition of comparative developmental data should help in determining the phylogeny of the Vitaceae. The shoot apex produces two tendril primordia (also called uncommitted primordia) at two nodes of a three-node cycle. Uncommitted primordia arise as follows: (1) a lower uncommitted primordium, opposite of a leaf, arises high upon the flank of the apex. (2) The upper uncommitted primordium, opposite of a leaf, arises above the leaf of the lower tendril node, low on the flank of the apex. Tendrils of *P. quinquefolia* generally bear 7 lateral branches whereas tendrils of *P. tricuspidata* generally bear 9 lateral branches. Tendril growth is clearly sympodial with terminating branches originating as the main portion of a tendril apex and the smaller portion of the apex is found in the axil of the bract. Tendril tips always bear adhesive pads: those pads of *P. tricuspidata* seem to arise precocially as swellings at the branch tips while those pads of *P. quinquefolia* only arise during later development. Epidermal cells at branch tips differ from stalk cells earlier in development. Upon contact with substrate, cells grow out from the surface and can make numerous divisions that increase the area of the surface.

**Roger F. Horton**
Department of Botany, University of Guelph, Guelph, ON, N1G 2W1, rhorton@uoguelph.ca

**Making ethylene**

Ethylene gas has been implicated as a co-regulator in a very wide range of growth and developmental phenomena. In higher plants, ethylene is biosynthesized in a pathway from methionine to 1-aminocyclopropane -1-carboxylic acid (ACC) which is oxidized to ethylene. Two key enzymes, ACC synthase and ACC oxidase have been characterized. In mosses, ferns and liverworts – which synthesize ethylene and can exhibit clear examples of ethylene responsiveness – the biosynthetic pathway remains an enigma. The ACC-dependent pathway is apparently not operational in these 'lower' plants, and the substrate(s) for ethylene production remain unknown.

Although the ACC-dependent pathway was presumed to be universal in angiosperms, there is evidence that *Potamogeton pectinatus* is incapable of synthesizing the gas. Work with two species of the submerged heterophyllous aquatic, *Cabomba*, suggests that the highly-dissected leaves do not synthesize ethylene from ACC, while the small, obovate leaves formed underwater on flowering shoots can evolve ethylene from this pathway. These preliminary results are discussed in terms of (a) the requirement for ethylene in plants (b) the occurrence and role of ethylene in submerged plants – particularly those with highly-dissected leaves and reduced vascularization and (c) the evolutionary position of *Cabomba* (based on developmental and molecular criteria) as a modern representative of a primitive angiosperm.

GUEST SPEAKER:

**Krzysztof Szczyglowski** and Lisa Amyot
Agriculture and Agri-Food Canada, SCPFRC, London, ON, N5V 4T3, szczyglowskik@em.agr.ca

**Symbiotic and non-symbiotic development in *Lotus japonicus***

The formation of nitrogen fixing nodules on legume plants in response to rhizobial infection is a complex developmental process. Upon infection with a specific strain of rhizobia, root cortical cells of legume plants undergo dedifferentiation, initiate cell divisions, and redirect their developmental fate towards the formation of nodule primordia. A highly controlled and organized series of events thereafter culminate in the formation of fully functional, nitrogen fixing organ, the root nodule. Despite the highly symbiont-specific and localized nature of nodule ontogeny, genetic data strongly suggest a distinct connection between nodule morphogenesis and other plant developmental processes.

The presentation will describe two examples of developmental processes involved in both symbiotic and non-symbiotic development in *Lotus japonicus*. First, a regulatory feedback loop that limits nodulation and preserves the homeostasis of the symbiotic interaction will be discussed. Secondly, an example of developmental control of the *PITP-IV* gene, encoding a novel type of phosphatidylinositol transfer protein in *L. japonicus* nodules and flowers, will be presented.

# Poster Presentation Abstracts

Poster Board #1

**Sajjad R. Akhter** and Roger S. Pearce
Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, SRAKHTER@HOTMAIL.COM

## *adsr36*, a sequence highly expressed in plants acclimating to abiotic stress

There are few reports of genes which are expressed in response to only one stress. A cDNA clone from *Agropyron desertorum* a drought- and cold-tolerant wheatgrass which can hybridise with wheat, was isolated and named as *adsr36* (*Agropyron-desertorum-stress-responsive*) gene. This gene is encoding a very hydrophobic protein with potentially two transmembrane domains, suggesting a possible role in maintaining the integrity of the membrane during dehydrative stress. Related sequences from other species have been suggested to be expressed in response to just one type of stress. For example, in barley it is reported as cold-responsive but in a wild salt-tolerant species, *Lophopyrum elongatum*, it is expressed in roots in response to salt. The expression of *adsr36* was observed in leaves, roots and meristems in different wild and cultivated cereals differing in adaptation to different stresses. *A. desertorum* expressed *adsr36* strongly in response to cold, moderately strongly in response to drought, and detectably in response to salt. However, *L. elongatum*, which is more salt-tolerant, did not express it highly in response to salt, and expressed it more strongly in response to cold and drought. Among the wheat cultivars, a strong signal was observed under cold stress, and signal under drought stress was second, but there were varietal differences in expression in response to other stresses. Thus, though the gene is strongly expressed during cold-acclimation, it is also often expressed during acclimation to drought. However, there is less indication of an association with acclimation to salt.

Poster Board #2

**Lisa Amyot**[1], Rebecca Cowan[2], Krissy Boucher[2], Clint Ali[2], and Krzysztof Szczyglowski[1]
[1]Agriculture and Agri-Food Canada, SCPFRC, London, ON, amyotl@em.agr.ca
[2]University of Western Ontario, London, ON, N6A 5B8

## A model legume, *Lotus japonicus*

Legumes are more than just a valuable source of proteins and edible oils. Many species of the family are valued for their medicinal and ornamental properties and some are being used in the production of dyes and timber. In addition to their economic importance, legumes are a significant source of natural fertilizer and contribute to the global nitrogen cycle. This family is also noticed for its almost unique ability in nature to interact with two major classes of symbiotic soil microorganisms, namely, nitrogen-fixing bacteria commonly known as rhizobia, and phosphate-acquiring arbuscular mycorrhizal fungi. It is these symbiotic relationships that have led to the natural emergence of legumes as a unique model system for studying the molecular basis of root-associated symbiotic interactions.

Our presentation will describe one of the currently developed model legume systems, *Lotus japonicus*. Like the model plant *Arabidopsis thaliana*, *Lotus japonicus* is a diploid, self-fertile plant with a relatively small genome and short generation time. This poster will illustrate several biological and genetic properties of this legume species and also highlight the advantages of using *Lotus japonicus* as an experimental model organism.

Poster Board #3

**Deborah L. Buhlers**, Barbara A. Moffatt, and Marilyn Griffith
Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, dbuhlers@hotmail.com

**Transformation with *CHT46* encoding a winter rye antifreeze protein does not enhance the freezing tolerance of *Arabidopsis***

At subzero temperatures, freezing-sensitive crop varieties are injured by ice crystal growth in extracellular spaces that leads to dehydration stress and mechanical damage within the tissues. Cold-acclimated winter rye (*Secale cereale*) survives freezing, in part by producing six dual-function antifreeze proteins that modify the growth of ice. A cDNA encoding a 24.9 kDa class II chitinase-antifreeze protein was isolated and transferred into *Arabidopsis thaliana* using *Agrobacterium*. Four *CHT46*-transformed *Arabidopsis* lines were characterized by measuring growth rate, protein activity and freezing tolerance. There were no significant differences between *CHT46*-transformed *Arabidopsis* lines and wild type in dry weights, number of leaves and freezing tolerance when these plants were grown at either 20°C or 5°C. Furthermore, no antifreeze activity was detected in whole cell extracts from the leaves of cold-acclimated and non acclimated *CHT46*-transformed or wild-type *Arabidopsis*, although increased chitinase activity was detected in several lines. The lack of antifreeze activity in *CHT46*-transformed *Arabidopsis* could be attributed to differences in protein folding or to problems in processing the apoplastic targeting sequence. Another possible explanation may be that *CHT46* requires an endogenous cofactor for antifreeze activity that is not present in *Arabidopsis*.

Poster Board #4

**R.J.N. Emery**[1] and C. Salon[2]
[1]Biology Department, Trent University, Peterborough, ON, K9J 7B8, nemery@trentu.ca
[2]Institut National de la Recherche Agronomique, Unité de Malherbologie et Agronomie, 17 Rue Sully, BV 1540, 21034 Dijon CEDEX, France

**Apparent aquaporin mediated water entry into pressurised detached root systems of pea, soybean and tomato follows saturating facilitated diffusion kinetics**

Although evidence is accumulating that water entry into roots occurs largely by a route regulated by aquaporins, flow is still widely modelled based on principles of ordinary passive diffusion. However, aquaporins are transporter proteins that facilitate water flow, so it will be a process that saturates as the number of functional aquaporins limits it. Using a pressure chamber system we observed pressure imposed flow saturation in peas, soybean and tomato, consistent with an aquaporin limited route of water flow. Therefore, a model based on facilitated diffusion and following Michaelis-Menten formalism was proposed. Experiments revealed that the plateau in flow, $J_{max}$ seen at high pressures followed natural rhythms diurnally and developmentally, and was not due to root damage or unnatural flow restriction. Evidence supports the hypothesis that diurnal rhythms in $J_{max}$ and conductivity, $L_p$, are a result of changes in aquaporin protein turnover. Interestingly, in the legumes, pea and soybean, transpirational demands were not met by $J_{max}$, which indicated that pressure induced water flow through isolated roots does not always reflect that of an intact transpiring plant. There is a possibility that the lower aquaporin flow in legumes may reflect their more conservative water usage.

Poster Board #5

**M.A. Fieldes** and C.G. Harvey
Department of Biology, Wilfrid Laurier University, Waterloo, ON, N2L 3C5, mfieldes@mach1.wlu.ca

## Growth and development of the 5-azacytidine-induced early flowering lines of flax (*Linum usitatissimum*)

The short-term treatment of germinating seed with the DNA-demethylating agent, 5-azacytidine, has been shown to induce heritable, epigenetic changes in growth and development in five plant species. In flax, the most interesting phenotypic change induced by the treatment was a marked reduction in flowering age. Three early flowering lines, derived from plants grown from seeds that were treated for 24 hours, are the focus of current research. At maturity, these lines are shorter than normal with fewer than normal leaves on the main stem. In each line, the early flowering phenotype is controlled by three independent loci. The model for the genetic control of early flowering has lead to a working concept of the roles of the genes, and how the genetic changes produce the early flowering phenotype. Recent work has provided information about two aspects of the working concept. Differences in hypocotyl development, between the early flowering and normal lines, indicate that the working concept may need to be adapted. Furthermore, the early flowering lines appear to be less phenotypically plastic than the normal lines, especially with respect to leaf number at maturity. In the early flowering lines, growing conditions that delay vegetative growth may delay flowering but rarely alter leaf number at maturity.

Poster Board #6

Brett J. Ferguson, Ryan D. Geil, **Frédérique C. Guinel**, Marie-Agathe Lorteau and Carolina M. Resendes
Department of Biology, Wilfrid Laurier University, Waterloo, ON, N2L 3C5, fguinel@wlu.ca

## Roles of plant hormones in the regulation of symbiotic structures in pea

Creating a diagram, we have dissected indeterminate nodule development into several steps. Many genes known to participate in nodule organogenesis were incorporated into this model. Each step was established based on studies of different pea lines, each with a single-gene recessive mutation that regulates a specific event in nodule development. This model is used to:

1) Better understand the processes involved in nodule organogenesis. Our model illustrates two main programs during nodule formation – an epidermal one and a cortical one. Although these two programs are independent of each other (e.g. the formation of pseudonodules does not share any events with the epidermal program), strong molecular links exist between them (e.g. for the formation of pre-infection threads).

2) Compare the developmental events between nodulation and arbuscular mycorrhizae formation. These symbioses share common steps, especially at pre-infection stages; the epidermis appears to play an important role in allowing entry of the microsymbiont into the root cortex.

3) Unravel roles of ethylene in these two symbioses. Generally, this hormone is inhibitory; it appears to act at several stages during the establishment of the symbioses. Direct effects of ethylene on nodulation include reduction of rhizobial infection number and positioning of nodule primordia in the inner cortex.

Poster Board #7

**Christopher Kozela**[1], Björn Sundberg[2], and Sharon Regan[1]
[1]Department of Biology, Carleton University, Ottawa, ON, K1S 5B6, ChrisKozela@pigeon.carleton.ca
[2]Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden

## How wood *Arabidopsis* do it?: Microarray analysis of developing *Arabidopsis* hypocotyls and the pursuit of control mechanisms in secondary xylem fiber formation

The molecular genetic controls governing wood formation are poorly understood. Advances in large-scale genomic approaches can identify genes of interest with regards to specific developmental programs. Extensive secondary growth in the hypocotyl of *Arabidopsis thaliana* allows this organism to be used as a developmental model of xylem formation. A cDNA microarray experiment was performed to identify genes of potential importance during secondary xylem development, both in maturing tissues as well as in a mutant (*ein4*) background that lacks xylem fiber production. Out of a possible 10,000 genes included in this analysis, only 84 and 53 genes displayed a significant change in transcriptional activity in the maturing and mutant conditions respectively. A significant number (~20) of these genes were found to be shared between the two experiments and included genes potentially involved in signal transduction, cell wall biosynthesis, hormone perception, transcriptional regulation, and cell death. Among these, further investigations will concentrate on determining the function of a subtilisin-like serine protease. A large number of genes were also identified to which no putative function could be proscribed. Large-scale identification of genes of interest allows for the implementation of reverse genetic techniques to determine specific gene function as well as the identification of gene networks regulating complex developmental programs.

Poster Board #8

**Hugues B. Massicotte**[1], Linda E. Tackaberry[1], Lewis H. Melville[2], and R. Larry Peterson[2]
[1] Forestry Department, University of Northern British Columbia, Prince George, B.C., V2N 4Z9, hugues@unbc.ca
[2] Department of Botany, University of Guelph, Guelph, ON, N1G 2W1

## The morphology and anatomy of two mycoheterotrophic species in central British Columbia: Fungal specificity north of 50!

*Monotropa uniflora* and *Pterospora andromedea* were sampled from a sub-boreal forest ecosystem in central B.C. In both *Monotropa* and *Pterospora*, mycorrhizal root clusters belonging to the same plant were morphologically and anatomically similar. Scanning electron microscopy of the root surface of *M. uniflora* showed an abundance of mantle cystidia and the presence of intra and extramatrical crystals, presumed to be calcium oxalate, in the fungal mantle. In *P. andromedea*, surface hyphae of the mantle were extensively covered with globular accumulations, also presumed to be calcium oxalate. Light microscopic examination of sectioned material of both revealed intracellular penetration of epidermal cells via a penetration peg, characteristic of monotropoid mycorrhizas. These structural aspects were similar to those described in the literature. Phylogenetic analysis suggested all *M. uniflora* mycorrhizae nested within the Russulaceae. *P. andromedea* clustered uniquely with the *Rhizopogon subcaerulescens* group. Structural and molecular evidence suggest that *M. uniflora* and *P. andromedea* form mycorrhizal associations with a limited number of fungal species well into their northern range in western North America.

Poster Board #9

**Lisa McDonnell**[1], Sara Andersson[2], Björn Sundberg[2], Bernie Glick[3] and Sharon Regan[1]

[1]Department of Biology, Carleton University, Ottawa, ON, K1S 5B6, lmcdonne@chat.carleton.ca
[2]Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden
[3]Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1

**Are ethylene levels tightly regulated in vascular tissues? - Functional analysis of a plant-encoded ACC deaminase**

The immediate precursor of the plant hormone ethylene is 1-aminocyclopropane-1-carboxylic acid (ACC) which is converted to ethylene through the action of ACC oxidase. In bacteria, ACC can be diverted away from ethylene production by the action of ACC deaminase which converts ACC to α-ketobutarate. To date, there have been no reports of ACC deaminase activity in plants, although putative ACC deaminase homologs have been identified in poplar and *Arabidopsis*. From the poplar EST library, an ACC deaminase-like clone was isolated from the wood-forming tissues of the stem. Interestingly, the transcript for this "ACC deaminase" is very tissue specific in the stem, being almost exclusively found in the phloem and undetectable in the xylem. This is in sharp contrast to the expression pattern for ACC oxidase, which is found in the xylem but not the phloem. This would suggest that there is a tight regulation of ethylene levels in vascular tissues which could be a determining factor in xylem and phloem identity. We are currently using several approaches to determine whether we have identified a bona fide ACC deaminase and to challenge this theory on the role of ethylene in vascular tissues.

Poster Board #10

**Athena D. McKown**[1], Oriana Pegoraro[2], and David D. Cass[1]

[1]Department of Biological Sciences, University of Alberta, Edmonton, AB, T6G 2E9, twinflower@hotmail.com
[2]4 Keating Pt., St. Albert, AB

**Band plasmolysis in leaf epidermal cells of *Clivia miniata* (Amaryllidaceae)**

Leaf epidermal cells of *Clivia miniata* Regel have strongly thickened outer tangential walls, sizeable cuticles and epicuticular wax deposits, features associated with survival in hot, dry climates. These cells also exhibit band plasmolysis, shown using a variety of cytological methods, and is a feature that has not been previously associated with epidermis. Results were best obtained by staining with the membrane fluorochrome RH414 (Molecular Probes) and followed by treatment in solutions of 0.8 M mannitol. Membrane adherence was seen on a thin, wavy anticlinal partition between epidermal cells. Attempts to stain this portion of cell wall with lipid binding reagents such as berberine, a stain commonly used for examining root exodermis and endodermis, were unsuccessful. Use of the apoplastic tracer pyrenetrisulfonate (PTS) revealed that PTS is not fully mobile in the thickened portion of anticlinal wall adjacent to the site of membrane adherence. A xerophytic taxon exhibiting this phenomenon suggests that its leaf epidermal cells possess a barrier to apoplastic water movement, forcing water into the symplastic pathway where greater cytoplasmic control might be realized.

Poster Board #11

**Wenjing Tao** and Marilyn Griffith
Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, w2tao@sciborg.uwaterloo.ca

## Freezing tolerance of *Thellungiella Salsuginea* and cold-regulated gene expression

*Thellungiella salsuginea,* a wild crucifer, is able to survive in cold environments and thus provides a particularly suitable model for studying the mechanisms involved in cold adaptation. Freezing tolerance was assayed by the regrowth of intact plants following cold and drought treatments. The freezing tolerance increased from an initial -13 $^0$C to -17 $^0$C after plants were grown for14 days at 5 $^0$C. Freezing tolerance was also enhanced by 3 days of drought treatment followed by 7 days of cold acclimation. Because the accumulation of specific cold-induced proteins is thought to be required for freezing tolerance in *Arabidopsis*, several of the corresponding genes were characterised in *Thellungiella* by RT-PCR. Northern analyses indicated that *CBF*, the transcriptional activator for cold-regulated genes (*COR*) was induced within 20 min of transferring plants to 5 $^0$C, followed by accumulation of *COR* gene transcripts at about 2 h. However, both *CBF* and *COR* genes were weakly expressed in plants grown at 5 $^0$C that were more freezing-tolerant. One gene that showed enhanced expression at 5 $^0$C was SPS, encoding sucrose phosphate synthase. The correlation between the level of freezing tolerance and the accumulation of SPS indicates that the regulation of carbohydrate metabolism may be the key to understanding freezing tolerance in *Thellungiella.*

Poster Board #12

**Margarita I. Todorova**[1], Luiz A. Pereira[1], Barbara A. Moffatt[1], and Chris A. Makaroff[2]
[1]Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, mtodorov@sciborg.uwaterloo.ca
[2]Dept of Chemistry, Miami University OH

## Changes in the expression and the activities of SAHH and ADK in major plant organs

The expression and the activity of two enzymes associated with the activated methyl cycle have been analyzed in the organs of *Arabidopsis thaliana.* The first enzyme studied, *S*-adenosylhomocysteine hydrolase (SAHH), is essential for the regeneration of *S*-adenosylmethionine, the principal methyl donor in cells. The second enzyme investigated, adenosine kinase (ADK), is responsible for the conversion of adenosine (ado), a product of SAH hydrolysis, to AMP. Since ado accumulation leads to inhibition of SAHH and ultimately inhibition of methylation reactions, its activity is critical for normal plant development. My project is focused on learning how SAHH and ADK activities, transcript and protein levels change in the organs in relation to different SAM-dependant methylation activities. The expression patterns of each SAHH gene were determined, indicating a lack of expression of one SAHH gene in roots and leaves, and possibly different regulation of these genes. Protein levels were monitored by immunoblotting and they were in good agreement with SAHH and ADK enzyme activity measurements. Moreover, *in situ* hybridization and immunofluorescent detection have been used as a second approach to localize SAHH and ADK transcript and protein levels in the different tissues of the organs studied. The future directions will continue toward understanding the regulation of SAM metabolism and how plant cells monitor their changing methyl requirements.

Poster Board #13

**Kimberly Walsh** and Vojislava Grbic
Department of Plant Sciences, University of Western Ontario, London, ON, N6A 5B8

**The interaction of ART1 and ART2 with EMF in *Arabidopsis thaliana***

AERIAL ROSETTE 1 (ART1) and AERIAL ROSETTE 2 (ART2) are both genes required for delayed flowering in *A. thaliana*. EMBRYONIC FLOWER (EMF) is another gene that has been implicated in regulating the timing of flowering (Yang et al., 1995). Plants homozygous for the mutant emf allele quickly bypass the vegetative phase and enter the reproductive phase of growth. Previous studies have revealed that various late-flowering mutations, when present with emf mutant alleles have been able to delay the quick transition to the reproductive phase. The focus of this study was to examine the interaction between ART1, ART2, and EMF, by creating appropriate crosses and examining the phenotype of plants with the genotype ART2/ART2, emf/emf, ART1/ART1. The phenotype of these plants demonstrated that ART1 and ART2 act in the same pathway as EMF, with EMF being epistatic.

## Notes

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